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Original Article

Microbial Exposure Assessment in Sawmill, Livestock Feed Industry, and Metal Working Fluids Handling Industry

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Objectives: The objective of this study is to investigate the distribution patterns and exposure concentrations of bioaerosols in industries suspected to have high levels of bioaerosol exposure.
Methods: We selected 11 plants including 3 livestock feed plants (LF industry), 3 metal working fluids handling plants (MWFs industry), and 5 sawmills and measured total airborne bacteria, fungi, endotoxins, as well as dust. Airborne bacteria and fungi were measured with one stage impactor, six stage cascade impactor, and gelatin filters. Endotoxins were measured with polycarbonate filters.
Results: The geometric means (GM) of the airborne concentrations of bacteria, fungi, and endotoxins were 1,864, 2,252 CFU/m^3, and 31.5 EU/m^3, respectively at the sawmills, followed by the LF industry (535, 585 CFU/m^3, and 22.0 EU/m^3) and MWFs industry (258, 331 CFU/m^3, and 8.7 EU/m^3). These concentrations by industry type were significantly statistically different ($p < 0.01$). The ratio of indoor to outdoor concentration was 6.2, 1.9, 3.2, and 3.2 for bacteria, fungi, endotoxins, and dust in the LF industry, 5.0, 0.9, 2.3, and 12.5 in the MWFs industry, and 3.7, 4.1, 3.3, and 9.7 in sawmills. The respiratory fractions of bioaerosols were differentiated by bioaerosol types and industry types: the respiratory fraction of bacteria in the LF industry, MWF industry, and sawmills was 59.4%, 72.0%, and 57.7%, respectively, and that of fungi was 77.3%, 89.5%, and 83.7% in the same order.
Conclusion: We found that bioaerosol concentration was the highest in sawmills, followed by LF industry facilities and MWFs industry facilities. The indoor/outdoor ratio of microorganisms was larger than 1 and respiratory fraction of microorganisms was more than 50% of the total microorganism concentrations which might penetrate respiratory tract easily. All these findings suggest that bioaerosol in the surveyed industries should be controlled to prevent worker respiratory diseases.

Key Words: Bioaerosols, Biological agents, Microbial risk, Sawmill, Livestock feed industry, Endotoxin

Introduction

Recently, biological agents in occupational environments have become social-issues due to increasing rates of occupational diseases. It is known that workers who are exposed to bioaerosols, such as farmers, sawmill workers, and those who handle metal working fluids, are associated with a wide range of adverse health effects, including infectious diseases, acute toxic effects, allergic reactions, and cancers [1]. In South Korea, occupational disease from bioaerosols has become the third most common worker's occupational disease following by pneumoconiosis and hearing loss. Despite the importance of exposure, risk assessment of bioaerosols is difficult because standard quantitative assessment methods have not yet been established and a field survey of occupational exposure levels to bioaero-

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sols has not been performed. Studies on microbial exposure assessments of indoor air are being conducted frequently to compare to regulated bioaerosols exposure limits to determine indoor air quality in public facilities (e.g. hospitals, subway stations, daycare centers). A total airborne bacteria of 800 CFU/m^3 is the limit in South Korea, but highly contaminated work environments have rarely been studied, so such studies are required to investigate exposure levels. There are many occupational activities which expose workers to high levels of microorganisms. First, it is well known that microorganisms easily develop in wood and, during wood processing, microorganisms are released into the air and high concentrations of airborne microorganisms may be present inside sawmills. Airborne microorganisms were identified as a cause of occupational pulmonary disease for woodworkers. Alwis et al. [2] reported that the prevalence of cough, phlegm, chronic bronchitis, nasal symptoms, frequent headaches, and eye and throat irritations was significantly higher among woodworkers than the control group.

Douwes et al. [3] reported that in large-scale industrial composting plants, since exposures to microorganisms and organic dust in the compost industry can be substantial, increasing the number of workers and occupational exposures of those compost workers may cause acute and possibly chronic inflammatory reactions in their upper airways, presumably induced by non-allergic pro inflammatory agents like endotoxins and (1-3)-β-D-glucans. Also, metal working fluids are extensively used in the metal working industry to cool and lubricate tool-workpieces, and workers exposed to aerosols of metal working fluids (MWFs) have been found to have more respiratory symptoms, including cough, phlegm, and chest tightness than the control group [4,5].

In order to prevent workers health problems and to establish quantitative assessment methods and occupational exposure limits of bioaerosols, knowledge of the workplace airborne contaminant concentration is necessary.

The objective of this study is to investigate microbial exposure assessment methods in locations known as work environments that are highly contaminated with microorganisms, such as sawmills, livestock feed industry facilities, and metal working fluids handling industry facilities, to evaluate the risk of microorganism contamination and related environmental parameters to prevent respiratory diseases in workers.

Materials and Methods

Target industry

The study investigated the bioaerosols and related factors in 11 industrial plants: 3 livestock feed plants, 3 metal working fluids handling plants, and 5 sawmills. As controls, the air in the backyard of each workplace was measured. For livestock feed plants, we investigated 3 main processes, raw material input, pellet making, and packaging, during May 2009 to June 2009. For MWFs plants, we investigated 3 main processing areas, computer numerical control (CNC) lathe (enclosure type), multi-functional manual (performing 9-task at a time, open-air type), and tapping center (enclosure type), during June 2009 to July 2009. For sawmills, we measured 3 main processes, band-sawing, table-sawing, and rip-sawing, in 2 hard-tree sawmills and 3 soft-tree sawmills during April 2009 to Sempember 2009.

Sampling design and method

The area samples were obtained in triplicate in each plant's 3 main processes as well as outdoors for one day while the processes were performed. We measured culturable microorganism with single-stage and six-stage anderson samplers and a filtration method, and endotoxin and dust (total dust for feed, oil mist for MWFs, and inhalable dust for wood dust) were measured with a filtration method. A single stage anderson sampler was used to investigate the total concentration, the six stage anderson sampler was used to investigate the size distribution of microorganisms, and the filtration method was used to compare the sampling method with two other impaction methods. But, in this journal we will show the correlation among them and, in another paper, we will clearly explain the comparison results. The impaction method was sampled 3 times a day for about 5 minutes each. The filtration method and endotoxins were sampled for about 6 hours with 3 parallel samples at one place. All air sampling pumps were calibrated using a flow meter (Bios DC-Lite; SKC) and field blank samples were included for each day's sampling for quality control. Temperature and relative humidity were also measured at each sampling location.

Impaction

Samplers were placed 1.5 m above the floor surface and as close to the work site as possible. Three types of air samplers were used at each sampling site: single-stage-viable particulate impactor (Model Quick take 30, SKC Inc, USA), six-stage viable particulate cascade impactor (Model TE 10-800, Tsich-environmental, USA), and gelatin filter with personal air sampler (SKC, USA). Anderson samplers were operated at 28.3 Lpm for 5 minutes for the livestock feed industry and the metal working fluids handling industry, and 2 minutes for sawmills. Six-stage viable particulate cascade impactor was used to investigate microorganism size distributions by aerodynamic size; stage 1 (> 7.0 μm), stage 2 (4.7-7.0 μm), stage 3 (3.3-4.7 μm),

stage 4 (2.1-3.3 μm), stage 5 (1.1-2.1 μm), and stage 6 (0.65-1.1 μm). A strerilized media was placed in each stage of equipment and before starting each sampling operation, the exterior of the sampling equipment was sterilized with 70% ethyl alcohol in order to prevent contamination. The media after sampling was immediately sealed with a laboratory film as soon as it was taken out from the equipment to prevent contamination and it was transported to the laboratory under 4°C. Tryptic Soy Agar (TSA; Lot HK082185, Komed, Korea) supplemented with cycloheximide 500 mg/L was used for the isolation of total bacteria and Sabouraud Dextrose Agar (SDAc; Lot HK081199, Komed, Korea) supplemented with chloramphenicol 100 mg was used for the isolation of total fungi. TSA plates were incubated at 37°C for 24-48 hours and SDA plates were incubated at 25°C for 72 hours, then cultured colonies were counted. Counted colonies were corrected by using positive hole correction factors. After the counting colonies, sampled media were sent to commercial laboratory for indentification.

Filtration

Filtration samples were collected on 25-mm gelatin filters (SKC, USA) housed in button air sampler (SKC, USA) for about 6 hours at 2.0 Lpm, and sampled at 1.5 m above the floor as close to the work as possible. PC (Polycarbonate) filters were used as backing pads. Samples were delivered under 4°C and analyzed within 24 hours. Filters were extracted with saline solution (0.9% NaCl solution) and diluted to 1:10, 1:100, and 1:1,000 and spread in 100 μL of diluted solution on TSA/SDAc plates.

Endotoxin

Airborne endotoxins were collected on sterile 37 mm × 0.8 μm PC filters for about 6 hours at 2.0 Lpm and sampled at 1.5 m above the floor as close to the work as possible. Samples were stored under -20°C and analyzed within 72 hours. Filters were extracted with 5 mL of pyrogen-free water and sonicated in a water bath for 60 min after 1 min of vigorous rocking. Endotoxins were assayed with a quantitative kinetic chromogenic LAL method at 37°C with an automated microplate reader (Lonza, USA). CSE (Control standard endotoxin, Lot #0000107398, Lonza, USA) was used as a standard endotoxin and matching Lysate (Lot #JL019A, Lonza, USA) was utilized.

Total dust samples (Livestock feed dust)

Dust samples were collected on pre-weighted 37 mm × 5.0 μm PVC (Poly vinyl chloride) filters housed in horizontally positioned 3-piece cassettes for about 6 hours at 2.0 Lpm and sampled at 1.5 m above the floor as close to the work as possible. Filters were desiccated with silica gel for 24 hours and weighed. Filters were weighed together before and after sampling using a microbalance (10^{-6} g sensitivity, Model UMT2 Mettler Toledo, Switchland).

Metal working fluids (Oil mist)

Dust samples were collected on pre-weighed 37 mm × 2.0 μm PTFE (Poly tetra fluoroethylene) housed in horizontally positioned 3-piece cassettes for about 6 hours at 2.0 Lpm. Filters were desiccated in silica gel for 24 hours and weighed. Samples were stored under 4°C and analyzed within 72 hours. Filters were weighed together before and after sampling using a microbalance (10^{-6} g sensitivity, Model UMT2 Mettler Toledo, Switchland). After placing the filter in the filter funnel assembly connected to the vacuum, 10 mL of Tetrahydrofuran solution was poured down a funnel to extract the oil, and then the filter was removed to clean the metal screen. After the filters were dried, they were weighed again. We calculated MWFs oil mist by the difference between the filter weight of after sampling and after extracting.

Inhalable dust (Wood dust)

Inhalable dust samples were collected on pre-weighted 25 mm × 0.8 μm PVC filters housed in horizontally positioned IOM cassettes (SKC, USA) for about 6 hours at 2.0 Lpm. Filters were desiccated in silica gel for 24 hours and weighed. The cassettes used had 50% efficiency at a 100 μm diameter; their collection efficiency approximated the inhalable curve. Filters with equilibrated cassettes were weighed together before and after sampling using a microbalance (10^{-6} g sensitivity, Model UMT2 Mettler Toledo, Switchland).

Statistical analysis

The data were analyzed using SPSS (version 17.0). Exposure variables were tested to determine whether they were normally or log normally distributed. GM (Geometric mean) and GSD (Geometric Standard Deviation) were calculated according to the log-normal distribution of the data. ANOVA and Dunnett's multiple comparison analysis from the log transformed data were used to verify the statistical significance for investigating the differences of airborne microorganism concentrations between industries. The Pearson's correlation analysis test was used to understand the correlation between each different microorganism sampling method.

Results

Microorganism concentration by industry

Table 1 summarizes microbial concentrations measured by

Table 1. Microbial concentration by industry, GM (Range)

Industry	n	Concentration (CFU/m^3)		Endotoxin (EU/m^3)	Dust (mg/m^3)
		Bacteria	Fungi		
LFeed	27	535 (93-4,770)	585 (115-18,572)	22.0 (3.5-196.9)	0.58 (0.0-1.7)
MWFs	27	258 (21-13,032)	331 (50-3,918)	8.7 (3.2-63.1)	0.4 (0.1-1.1)
Sawmill	36	1,864 (106-21,173)	2,252 (108-30,954)	31.5 (4.6-144.6)	2.4 (0.2-40.6)
F-value		21.758	16.831	71.506	15.625
p-value		< 0.001	< 0.001	< 0.001	< 0.001

GM: geometric mean, n: sample numbers, CFU: colony forming unit, EU: endotoxin unit, LFeed: Livestock Feed, MWFs: metal working fluids handling industry.

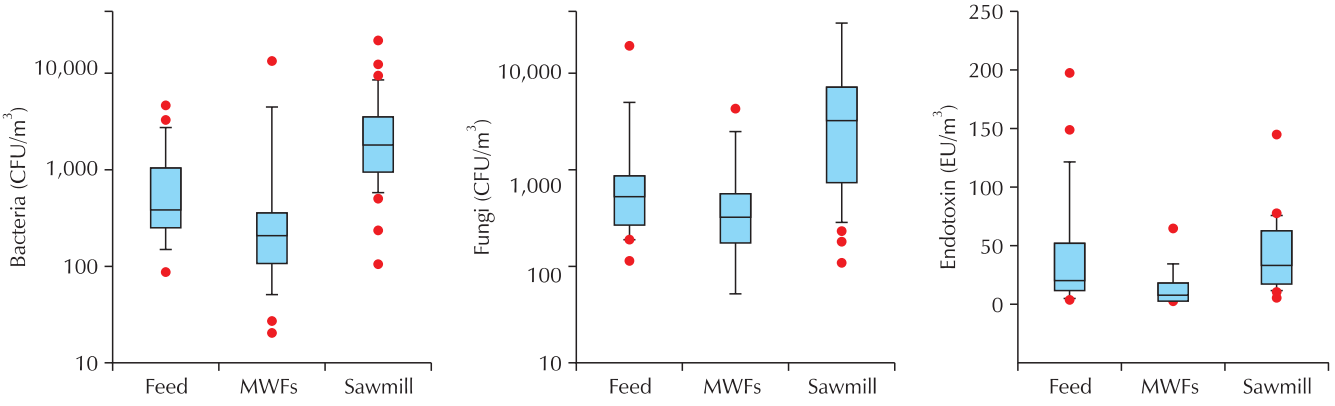

Fig. 1. Microbial concentration by industry, The boxes show the 25th and 75th percentiles and whiskers indicate 10th and 90th percentiles. Median is indicated by the line inside the box. CFU: colony forming unit, EU: endotoxin unit, MWFs: metal working fluids handling industry.

single stage anderson impactor. Total airborne bacteria concentration (GM, Range) in the LF industry, MWFs industry, and sawmills was 535 (93-4,770), 258 (21-13,032), and 1,864 (106-21,173) CFU/m^3, respectively, and total airborne fungi concentration (GM, Range) in each industry was 585 (115-18,572), 331 (50-3,918), and 2,252 (108-30,954) CFU/m^3, respectively. The results show that the total airborne bacteria and fungi concentration at the LF industry and MWFs handling industry facilities were not significantly different, but the concentration at the sawmills was significantly higher than the other industries ($p < 0.001$). The endotoxin level was 31.5 EU/m^3 at the sawmills, 22.0 EU/m^3 at LF industry facilities, and 8.7 EU/m^3 at MWFs industry facilities. The results show that the endotoxin concentrations for the sawmills and LF industry facilities were significantly higher than that of the MWFs industry facilities ($p < 0.001$).

Comparing these concentrations with recommended exposure limits for indoor air (800 CFU/m^3, Korean Ministry of Labor) and temporary exposure limits for occupational environments (10,000 CFU/m^3, German) (Fig. 1), 80.5% (29/36) of bacteria samples and 86.2% (25/29) of fungi samples from sawmills exceeded the indoor air limits and 2 bacteria samples and 6 fungi samples exceeded occupational limits. 29.6% (8/27) of bacteria samples and 25.9% (7/27) of fungi samples from the LF industry exceeded indoor air limits and 2 fungi samples exceeded occupational limits. 14.8% (4/27) of bacteria samples and 18.5% (5/27) of fungi samples from the MWFs industry exceeded indoor air limits and 2 bacteria samples exceeded occupational limits.

The most abundant genera of bacteria in the LF industry were *Bacillus subtilli, Klebsiella pnemonae,* and *Aerococcus viridians*. In MWFs industry, *Micrococcus luteus*, *Moraxella sp.*, and *Sphingmonas paucimobilis* were the most abundant genera of bacteria. In sawmills, *unidentified gram (+) bacillus* and *Sphingmonas paucimobilis* were detected the most. The most abundant genera of fungi were *Cladosporium ap., Penicillium sp.*, and *Aspergillus sp.* in the LF industry and MWFs industry. But, in sawmills, unidentified *moldform* was detected the most.

Table 2 shows microbial concentrations in various processes in the LF Industry. The results shows that fungi and

Table 2. Microbial concentration in livestock feed industry by processes

Process	n	GM (Range)					
		Bacteria (CFU/m^3)	Fungi (CFU/m^3)	Endotoxin (EU/m^3)	Dust (mg/m^3)	Temperature	Humidity
Overall	36	338 (14-4,770)	487 (115-18,572)	16.4 (3.3-196.2)	0.44 (0.0-1.7)	27.3 (23.1-34.2)	49.1 (32-64)
Raw matrial	9	326 (93-1,028)	953 (115-18,572)	29.2 (13.3-62.9)	0.9 (0.2-1.7)	25.4 (23.1-26.9)	57.0 (48-64)
Pelleting	9	861 (258-3,379)	428 (212-971)	18.4 (3.5-196.9)	0.6 (0.3-1.0)	30.6 (25.3-34.2)	44.3 (32-58)
Packaging	9	545 (144-4,770)	491 (182-847)	19.8 (6.3-65.4)	0.4 (0.0-1.7)	27.6 (26-28.7)	50.3 (44-58)
Outdoor	9	85.7 (14-459)	281 (144-850)	6.8 (3.3-12)	0.2 (0.1-1.1)	26.0 (25.2-27.2)	45.9 (38-52)
F-value		8.301	2.196	3.489	2.663	17.158	5.353
p-value		< 0.001	0.108	0.027	0.065	< 0.001	0.004

GM: geometric mean, n: sample numbers, CFU: colony forming unit, EU: endotoxin unit.

Table 3. Microbial concentration in metal working fluids handling industry by processes

Process	n	GM (Range)					
		Bacteria (CFU/m^3)	Fungi (CFU/m^3)	Endotoxin (EU/m^3)	Dust (mg/m^3)	Temperature	Humidity
Overall	36	185 (14-13,032)	338 (50-3,918)	7.0 (3.2-63.1)	0.4 (0.1-1.1)	26.6 (21.8-29.2)	58.1 (48-72)
CNC	9	116 (28-368)	122 (50-544)	8.4 (3.2-33.4)	0.2 (0.1-0.3)	23.8 (21.8-25.0)	58.2 (53-66)
Manual	9	669 (130-13,032)	277 (167-442)	9.4 (3.3-63.1)	0.5 (0.3-1.1)	27.6 (26.2-29.0)	66.9 (62-72)
Tapping center	9	222 (21-2,272)	1,074 (182-3,918)	8.4 (3.2-33.4)	0.7 (0.5-1.1)	28.6 (28.4-29.2)	53.2 (48-59)
Oudoor	9	51.4 (14-137)	365.6 (182-739)	3.8 (3.2-6.4)	0.04 (0.0-0.1)	26.6 (24.7-28.5)	53.9 (49-61)
F-value		5.998	12.459	2.073	7.999	30.723	16.754
p-value		0.003	< 0.001	0.125	< 0.001	< 0.001	< 0.001

GM: geometric mean, n: sample numbers, CFU: colony forming unit, EU: endotoxin unit, CNC: computerized numerical control.

Table 4. Microbial concentration in sawmill by processes

Process	n	GM (Range)					
		Bacteria (CFU/m^3)	Fungi (CFU/m^3)	Endotoxin (EU/m^3)	Dust (mg/m^3)	Temperature	Humidity
Overall	48	1,343 (53-21,173)	1,580 (42-30,954)	22.9 (4.1-144.5)	1.4 (0.1-40.6)	24.0 (20.3-28.9)	38.1 (21-71.5)
Band saw	12	3,326 (706-21,173)	2,750 (289-30,954)	43.1 (12-144.5)	2.1 (0.2-40.6)	24.0 (20.3-27.1)	37.8 (23-67)
Table saw	12	2,336 (904-9,100)	1,637 (108-30,954)	30.7 (9.6-65.5)	1.3 (0.4-3.5)	23.4 (20.3-26.7)	39.9 (23-71.5)
Rip saw	12	834 (106-3,922)	2,537 (289-30,954)	23.1 (4.6-65.4)	5.4 (1.8-19.5)	24.1 (20.3-26.7)	38.3 (23-70.3)
Outdoor	12	503 (53-7,123)	547 (42-5,261)	9.5 (4.1-28.7)	0.3 (0.1-1.1)	24.8 (21.6-28.9)	36.6 (21-63.6)
F-value		8.564	2.577	8.446	2.694	0.933	0.137
p-value		< 0.001	0.066	< 0.001	0.074	0.433	0.937

GM: geometric mean, n: sample numbers, CFU: colony forming unit, EU: endotoxin unit.

dust levels were not significantly different among the processes (fungi; p = 0.108, dust; p = 0.065). But, the bacteria and temperature at the pelleting process location and the endotoxins and humidity at the raw material process location exhibited the highest concentration among the processes. The outdoor concentrations were significantly lower than the indoor workplaces (p < 0.05). I/O (indoor/ outdoor concentration) ratio of each group's GM was 6.2 (Range, 4.2-9.5), 1.9 (Range, 1.3-3.3), 3.2 (Range, 2.1-5.0), and 3.1 (Range, 2.2-4.7) for bacteria, fungi, endotoxins, and dust, respectively.

Table 3 shows microbial concentrations among the various processes in the MWFs Industry. The results show that endotoxin levels were not significantly different among the processes. Bacteria at the manual process location exhibited the highest concentration among the processes and the fungi and oil mist levels at the tapping center process location were significantly higher than those of other processes (p < 0.001). I/O (indoor/outdoor concentration) ratio of each group's GM was 5.0 (Range, 2.8-9.2), 0.9 (Range, 0.6-1.5), 2.3 (Range, 1.5-3.4), and 12.5 (Range, 7.5-13.3) for bacteria, fungi, endotoxin, and dust, respectively.

Table 4 shows microbial concentraions among the various processes in the sawmill industry. The results show that the fungi, dust, temperature, and humidity were not significantly different among processes. But, bacteria and endotoxin levels at the band-saw and table-saw locations were significantly higher than those of other processes (p < 0.05). I/O (indoor/outdoor concentration) ratio of each group's GM was 3.7 (Range, 2.6-5.3), 4.1 (Range, 2.4-7.0), 3.3 (Range, 2.5-4.4), and 9.7 (Range, 4.2-22.2) for bacteria, fungi, endotoxin, and dust, respectively.

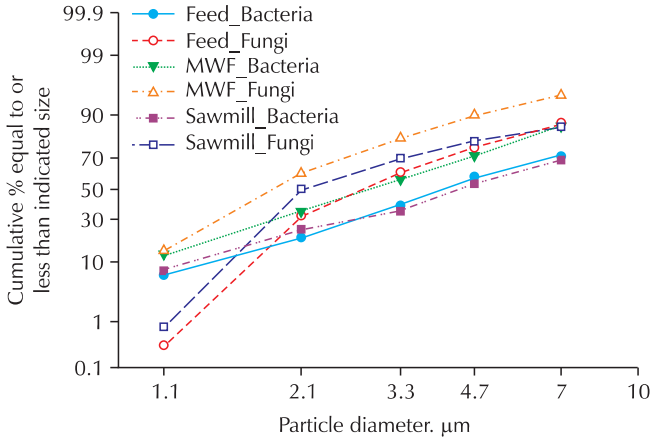

Fig. 2. Size distribution of cultured airborne bacteria and fungi according to six stage anderson sampler's each stage cut point. MWF: metal working fluids handling industry.

Size distribution of cultured microorganism

To investigate the size distribution of airborne bacteria, we divided six stages into two groups; one was the non-respirable microorganism size (> 4.7 μm) of stage 1 to stage 2 and the other was the respirable dust size (< 4.7 μm) of stage 3 to stage 6. By industry, respirable microorganism size constituted 59.4% for the LF industry, 72.0% for the MWFs industry, and 57.7% at sawmills. For the size distribution of fungi, respirable dust size constituted 77.3% for the LF industry, 89.5% for the MWFs industry, and 83.7% at sawmills (Fig. 2). The MWFs industry had the largest respirable microorganism size among the work sites and fungi had higher respirable fraction distributions than bacteria.

Correlation among microorganism sampling methods

The results of correlation analysis between microorganims showed that endotoxin and bacteria concentrations are strongly

Table 5. Size distribution of microorganism by industry (%)

Industry	Process	Bacteria (%)		Fungi (%)	
		Non-resp. (1-2 stage)	Resp. (3-6 stage)	Non-resp. (1-2 stage)	Resp. (3-6 stage)
LF industry	Overall	40.6	59.4	22.7	77.3
	Raw material	43.4	56.6	22.8	77.2
	Pallet	42.7	57.8	31.9	68.1
	Package	38.1	61.9	14.8	85.2
	Outdoor	35.9	64.9	22.0	78.0
MWFs industry	Overall	28.0	72.0	10.5	89.5
	CNC	13.0	87.0	22.1	77.9
	Manual	27.7	72.3	13.0	87.6
	Tapping	53.1	46.9	7.5	92.5
	Outdoor	38.9	61.1	19.6	80.4
Sawmill	Overall	42.3	57.7	16.3	83.7
	Band-saw	32.0	68.0	21.3	78.7
	Table-saw	49.1	50.9	9.2	90.8
	Rip-saw	50.8	49.2	12.5	87.5
	Outdoor	54.9	45.1	15.2	84.8

Non-resp.: particulate size higher than 4.7 μm, Resp.: particulate size lower than 4.7 μm, LF: Livestock Feed, MWFs: metal working fluids handling, CNC: computerized numerical control.

Table 6. Correlation among microorganism exposure methods

Type		Bacteria			Fungi			Endotoxin	Dust
		1 stage	6 stage	Filter	1 stage	6 stage	Filter		
Bacteria	1 stage	1	0.628†	0.093	0.147	0.175	0.293†	0.263†	0.063
	6 stage		1	0.115	0.008	0.021	-0.017	0.228*	0.112
	Filter			1	-0.010	-0.036	0.114	0.601†	0.152
Fungi	1 stage				1	0.818†	0.031	0.143	0.042
	6 stage					1	0.234*	0.200*	0.436†
	Filter						1	0.193	0.270*
Endotoxin								1	0.490†
Dust								1	

*$p < 0.05$,
†$p < 0.01$.

correlated with filtration methods (r = 0.601), weakly correlated with impaction methods (single stage r = 0.263, 6-stage r = 0.228), and correlated with dust (r = 0.490). The bacteria concentration from the single stage and 6-stage analysis have strong correlations (r = 0.628) and the fungi concentration from the single stage and 6-stage analysis have highly strong correlations (r = 0.818). But the bacteria concentration from the single stage and filtration analysis have no correlation (r = 0.093) and the fungi concentration from the single stage and filtration analysis also have no correlation (r = 0.031).

But in the LF industry, endotoxin and bacteria concentrations have strong correlations with all culture based methods (single stage, r = 0.661, 6-stage r = 0.623, filtration r = 0.612) and are weakly correlated with dust (r = 0.329). The bacteria concentration from the single stage was strongly correlated with the 6-stage and filtration analyses (r = 0.957 and 0.650, respectively) and the fungi concentration from the single stage analysis was also strongly correlated with the 6-stage analysis (r = 0.829), but it had no correlation with the filtration analysis (r = -0.047). In the MWFs industry, endotoxin and bacteria concentrations had strong correlations with the single stage stage analysis (r = 0.700, 6-stage r = 0.717) but exhibited no correlation with the filtration methods (r = -0.18), and was weakly correlated with dust (r = 0.358). The bacteria concentrations from the single stage and 6-stage analyses have a highly strong correlation (r = 0.960) and the fungi concentration from the single stage and 6-stage analyses have a highly strong correlation (r = 0.996). The bacteria concentration from the single stage and filtration analyses have no correlation (r = 0.146), but the fungi concentration from the single stage and filtration analyses exhibit a correlation (r = 0.533). In sawmills, endotoxin and bacteria concentrations have no correlation with the single stage (r = 0.072) and 6-stage (r = 0.290) analyses, but they do exhibit correlations with filtration methods (r = 0.565), and they are strongly correalted with dust (r = 0.890). The bacteria concentration from the single stage and 6-stage analyses have a highly strong correlation (r = 0.896), and the fungi concentration from the single stage and 6-stage analyses have a highly strong correlation (r = 0.835). The bacteria concentration of the single stage and filtration analyses have no correlation (r = 0.095), but the fungi concentration from the single stage and filtration analyses also have no correlation (r = -0.078).

Discussion

The results shows that sawmills had significantly higher total airborne bacteria and fungi concentrations than those of other industries ($p < 0.001$) and the endotoxin concentrations at sawmills and in the LF industry facilities were significantly higher than that in the MWFs industry ($p < 0.001$). We could see that microorganisms usually developed in the bark of a tree during the storage period and, while sawing the wood, microorganisms are released into the air and high concentrations of airborne microorganisms become exposed to the workplace. Some other studies on the microbial assessment of the wood industry are being conducted. Rongo et al. [6] investigated inhalable dust and endotoxin levels in 157 samples from 136 workers from small-scale wood industries in Africa (Tanzania).

Data showed a GM of 3.3 mg/m^3 (0.45-67.0 mg/m^3) for inhalable dust measured with a PAS-6 sampling head, a GM of 91 EU/m^3 (9-4,914.8 EU/m^3) for endotoxins measured with 5 mL Tween 20 extraction water and a LAL Assay, and the dust and endotoxin levels were weakly correlated ($r = 0.44$, $p < 0.0001$). The GM for inhalable dust was similar to our study concentration of 2.4 mg/m^3 (0.24-40.2 mg/m^3), but the endotoxin concentration was 3 times higher than our study concentration of 31.5 EU/m^3 (4.6-144.6 EU/m^3). Alwis et al. [2] investigated personal exposure levels to fungi, bacteria, and endotoxin at different woodworking sites. Exposure levels to fungi at logging sites and sawmills were in the range of 10^3-10^4 CFU/m^3, 10^3-10^5 CFU/m^3 at wood chipping mills, and 10^2-10^4 CFU/m^3 at joineries, although the mean endotoxin levels were lower than the suggested threshold value of 20 ng/m^3. Duchaine et al. [7] investigated seventeen sawmills in Canada with all-glass impingers and six-stage anderson microbial samplers and the debarking site was the working site most highly contaminated by molds, bacteria, and endotoxins. The median values for culturable bacteria and endotoxins were 21,620 CFU/m^3 and 1,081 EU/m^3, respectively. There was no debarking process in our study. Instead, the band-saw process site was the working site most highly contaminated by molds, bacteria, and endotoxins, but it exhibited a much lower concentrations compared with the debarking process site (3,326 CFU/m^3 and 43.1 EU/m^3 for culturable bacteria and endotoxin, respectively).

Kim et al. [8] investigated airborne bacteria and fungi in three feedstuff-manufacturing factories in Korea. The geometric mean (GM) levels of airborne bacteria and fungi were 113 (± 18) CFU/m^3 and 89 (± 5) CFU/m^3 for the pelleting process location and 198 (± 5) CFU/m^3 and 124 (± 12) CFU/m^3 for the powdering process location, respectively. This was a very low concentration compared with our study. In our study, bacteria and fungi at the pelleting process location were 861 CFU/m^3 and 428 CFU/m^3, respectively.

In our study, the MWF industry showed the lowest microorganism concentration among the studied industries and, compared with the indoor public facility bacteria exposure limit of 800 CFU/m^3, 14.8% of the bacteria samples from the MWF industry data exceeded the limit. The airborne microorganism concentration in the MWF industry can be affected by type of machine (automatic vs manual), oil (synthetic vs non-synthetic), ventilation (mechanical vs natural), and procedures (unifunctional vs multifunctional). Oh et al. [9] investigated 9 engine plants used by the MWFs industry. The gravimetric concentration of oil mist averaged 0.33 mg/m^3. The endotoxin concentration ranged from 14 to 644 EU/m^3 and the GM was 48.7 EU/m^3 in air. Airborne microbe concentrations were 38-42,500 CFU/m^3. But, Lee et al. [10] showed that, in a metal pipe cutting factory, the air exposure levels of oil mist, endotoxin, total bacteria, and fungi were TWA (8-hour) 0.531 mg/m^3, 6.33 EU/m^3, 100 CFU/m^3, and 75 CFU/m^3, respectively.

When the I/O ratio is larger than 1, we can infer that there was an inside contaminant source and a potential health hazard due to exposure of workers to the airborne microorganism. However there is a limitation to evaluating the degree of contamination based only on quantitative measurement values [11].

The proportion of respirable size of fungi (< 4.7 μm) was higher than that of fungi in the livestock feed industry and MWFs industry, and sawmills and the MWFs industry had the most respirable size in the work sites although they exhibited the lowest concentration among the industries. The proportion was much higher than the proportion of welding fume's respirable dust (66.1%) [12]. Based on a precedent study by Kim et al. [11], the proportion of microorganisms of respirable size in a feeding factory was very high and was more than 60% of the total microorganism concentrations; also, the study by Li and Kou [13] reported that the concentration of respirable fungi in the living rooms, bedrooms, and kitchens in apartments at six locations was 70-80% of the total microorganism concentrations. In a domestic precedent study by Kim et al. [8], the proportion of microorganisms of respirable size in public facilities was 55-70% of the total airborne bacteria concentrations and 50-60% of the total airborne fungi concentration.

We found that endotoxins had strong correlations with the filtration methods of bacteria and weak correlations with the impaction methods of bacteria in all industries. But it was different by industry. In the livestock feed industry, endotoxin and bacteria concentrations had strong correlations with all culture based methods. In the MWFs industry, endotoxins had no correlation with filtration methods. In sawmills, endotoxin and bacteria concentration had no correlation with single stage and 6-stage sampling methods. We can infer that airborne microorganism concentration can be affected by sampling methods and by different work environment conditions; the concentration also can fluctuate. Based on this information, we recommend that when sampling an airborne microorganism, one must consider which methods would be appropriate for the target industry and when comparing to other research results, one must notice the sampling and analytical methods.

The average coefficient of variance of bactria concentration based on the single stage sampling method was 56.5%, and based on the six stage sampling method it was 62.6%, and based on the filtration sampling method it was 51.0%. The average coefficient of variance of fungi concentration based on the single stage sampling method was 47.0%, based on the six

stage sampling method it was 50.8%, and based on the filtration sampling method it was 47.5%. The average coefficient of variance of endotoxin concentration was 27.2% and that of dust was 27.0%. Thus, endotoxins could be used as an adjustable assessment method due to their strong correlation with the filtration method (r = 0.601) and the lowest coefficient of variance in an occupational environment. We suggest that more research on how environmental parameters effect bioaerosol concentrations and intervention studies regarding these industries be performed in the future.

Conclusions

We found that bioaerosol concentration was the highest in sawmills, followed by LF industry facilities and MWFs industry facilities. The indoor/outdoor ratio of microorganisms was larger than 1 and respiratory fraction of microorganisms was more than 50% of the total microorganism concentrations which might penetrate respiratory tract easily. All these findings suggest that bioaerosol in the surveyed industries should be controlled to prevent worker respiratory diseases.

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